Letters in Animal Biology

Journal homepage: www.liabjournal.com

# Rotavirus prevalence with G and P genotypes circulated in different regions of India

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***Article info***
Received: 16 March 2025
Received in revised form: 07 April 2025
Accepted: 09 April 2025
Published online: 10 April 2025

**Keywords**
Rotavirus
Genotyping
PAGE
RT-PCR
Prevalence

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**Abstract**

Rotaviruses are widely acknowledged as the prime cause of severe gastroenteritis in children globally especially in under developed countries. This contemporary study presents data on the prevalence and genotypic distribution of human group A rotaviruses across various regions of India. 217 fecal samples were collected entirely from children and adults over 5 years of age from the Western (n=30), Southern (n=98), and North-Eastern Hill (NEH) (n=89) regions/zones of India and tested for the presence of rotaviruses. The RNA extracted from these stool samples was analyzed using Polyacrylamide Gel Electrophoresis (PAGE) and Reverse Transcription Polymerase Chain Reaction (RT-PCR). Rotaviruses were identified in 20.27% of the samples through PAGE and/or RT-PCR, with the highest detection rate of 41.37% observed in the Western region, followed by 17.34% in the Southern zone/ region, and 18.98% in the NEH region. A higher prevalence of 27.38% was observed in males than female children (14.06%), especially during winter (November to March) (34.84%) followed by April to June (15.62%). A total of fifteen samples were successfully typed for the G genotype, and six for the P genotype, through amplification and subsequent sequencing of *VP4* and *VP7*gene products. The prevalent G genotypes detected were G1, succeeded by G12 and G11, with one sample each also identifying G2 and G10. Amid P genotypes, P[6] was the most prevailing genotype, while P[4] and P[8] were each recognised in a single sample. The dominant genotype was G1P[6], with one sample showing a G1P[4] combination, and two samples exhibiting G11P[6] and G11P[8] combinations, which are rarely reported in India.



## 1. Introduction

Rotaviruses are widely endorsed as a vital cause of severe acute gastroenteritis and infant diarrhoea in children, animals, and birds internationally (Midgley et al. 2012; Malla et al. 2024). In 2015, diarrhoeal abnormalities were the fourth notable cause of death for children under 5 years old, accounting almost half a million fatalities (Troeger et al. 2018). A significant proportion, about 29.3%, of these deaths were due to rotavirus infections, with the majority occurring in low developing and middle-income countries (Parashar et al. 2016). In India alone, rotavirus causes roughly 11.37 million cases of acute gastroenteritis in little ones under 5 years of age each year, leading to a direct economic loss of INR 10.37 billion annually (John et al. 2014; Malla et al. 2025). Genome of rotavirus consists of 11 double-stranded RNA segments. Based on the genetic and antigenic diversity within the VP6 region, as well as on the basis of electrophoretic mobility of the RNA viral genome segments in RNA-PAGE, rotaviruses have been categorized into 10 groups or species (A–J). Recently, two additional groups, K (RVC-like) and L (RVH-like), have been identified. Groups A–C can cause infection in both animals and humans (Matthijnssens et al. 2012), whereas groups D–I are exclusively found in animals, particularly birds, with the exception of group E strains, which have been isolated only from pigs (Matthijnssens et al. 2011). Rotaviruses in group A are additionally categorized into G and P genotypes based on sequence variations in the VP7 and VP4 proteins. Currently, 58 well defined P genotypes (P[1]–[58]) and 42 G genotypes (G1–42) have been recognized, which are classified through nucleotide sequence analysis of the VP7 and VP4 genes, a process that can be achieved using semi-nested RT-PCR (https://rega.kuleuven.be/cev/viralmetagenomics/virus-classification2021/rcwg; Malla et al. 2024). The emergence of new

rotavirus strains is attributed to the ongoing mutation and reassortment of their double-stranded RNA (dsRNA) genome, which is segmented. The 11 dsRNA segments exhibit significant heterogeneity in gene sequences among different RV strains. Through complete genomic sequencing of both animal and human RVA strains, it has become evident that there are clear indications of repeated crossovers between the evolution of animal and human rotaviruses. These crossovers occur as a result of multiple instances of interspecies transmission followed by subsequent adaptation (Martella et al. 2010). The most prevalent P-types (P[4] and P[8]) and G-types (G1–4) globally account for around 3/4th of all rotavirus strains in the Indian subcontinent (Miles et al. 2012). In many studies from India, the most commonly identified strains include G1, G2, G3, G4, P [4], P[6], and P[8]. Nevertheless, another G-types for instance G6, G8, G9, G10, and G12 have too been linked to cases of diarrhoea (Ramani and Kang 2007). A notable number of children were found to have mixed rotavirus infections. While universally ubiquitous strains like G1P[8], G2P[4], G3P[8], and G4P[8] are accounting for just 33% of cases in Indian children, strains with P-type [6] (such as G1P[6], G2P[6], G3P[6], G4P[6], and G9P[6]), that typically affect asymptomatic infants but are less frequent in children accompanied by diarrhoea, were found in 43% of cases in India. Additionally, several atypical genotypes, including G1P[4], G2P[8], G2P[6], G4P[4], and G4P[6], were too observed (Das et al. 2002). Overall, G1P[8], G2P[4], G9P[8], G12P[6], and G12P[8] emerged as key G–P combinations in India.

Given the vast geographical area, diverse agro-climatic environmental conditions, and rich biodiversity of animals, humans, and microorganisms, understanding the circulating rotavirus genotypes is utmost important for evaluating the success of current rotaviral vaccines. It also plays a pivotal role in the advancement of new and more suitable vaccines to address rotaviral diarrhoea caused by various genotypes. Therefore, the focus of the present study was to explore the prevalence of group A rotaviruses based on sex, region and season, including the distribution of P and G genotypes in selected areas/regions of the country like India.

## 2. Materials and methods

### 2.1 Collection of faecal/stool samples

A total of 217 faecal/ stool samples, including 182 from diarrhoeal cases and 35 from non-diarrheal cases, were acquired from children less than 5 years of age and adults (above 5 years) from both private and government hospitals across different zones/regions of the country, with permission from the hospital authorities (Table 1). Informed consent was duly obtained from the caretakers of each child before any stool sample was collected (a non-invasive procedure). The stool samples were stored at cold temperature (-20°C) in the respective hospital laboratories. The samples were gathered from the Western region (Mumbai), the Southern region (Hyderabad, Bangalore, and Bidar), and the North Eastern Hill (NEH) region (Shillong and Gangtok). Subsequently, 20-30 samples were transported at various intervals on ice to the Veterinary Public Health laboratory at the Indian Veterinary Research Institute, Izatnagar, Bareilly, India, where they were kept at -20°C until further analysis.

### 2.2 Extraction of dsRNA from stool samples and detection by RNA-PAGE and RT-PCR

To detect rotaviruses in both diarrhoeal and non-diarrhoeal fecal samples, double-stranded RNA from infected faeces was extracted using the standard phenol-chloroform method (Sambrook and Russell 2001). The dsRNA was then analyzed through Polyacrylamide Gel Electrophoresis (PAGE) using a 7.5% gel as resolving gel and 5.0% gel as stacking gel, following the established methodology/technique (Herring et al. 1982), and stained with silver stain (Svensson et al. 1986). Reverse Transcription Polymerase Chain Reaction (RT-PCR) was performed to amplify partial gene segment 8 or 9 (*VP7* gene) from all group A rotaviruses (Hussein et al. 1993). The amplification products were assessed through electrophoresis on a 1.5% agarose gel harbouring 2 $\mu$L of ethidium bromide (10 mg/mL) in Tris-borate (TB) buffer, then visualized under a UV transilluminator, and documented using a gel imaging system.

### 2.3 P and G genotyping by RT-PCR

G genotyping was performed by amplifying the entire length *VP7* (1062 bp) gene using the method outlined by Gouvea et al. (1994a), followed by two semi-nested multiplex PCRs with multiple sets of specific primers for G genotype (first multiplex: 9T1-1, 9T1-2, 9T-3P, 9T-4, 9T-9B; second multiplex: FT5, DT6, HT8, ET10, BT11) (Das et al. 1994; Gouvea et al. 1994a) (Table 2). Likewise, P genotyping was conducted by amplifying a partial segment of the *VP4* gene [nucleotide (nt) position 11-887] as described by Gentsch et al. (1992), followed by two semi-nested multiplex PCRs by using different sets of specific primers for P genotype- (third multiplex: 1T-1, 2T-1, 3T-1, 4T-1; fourth multiplex: pUK, POSU, pNCDV, pB223) (Gentsch et al. 1992; Gouvea et al. 1994b) (Table 2). The resulting amplification products were examined via electrophoresis on a 1.5% agarose gel as previously elucidated.

### 2.4 Sequencing, sequence evaluation and phylogeny

Nucleotide sequencing of the *VP4* (P genotype) and *VP7* (G genotype) genes was outsourced to M/s. Chromus Biotech, Bangalore, India. Purified PCR products of the amplified *VP4* and *VP7* genes were provided along with the related primers to sequencing company. The sequences obtained were analyzed using NCBI BLAST (http://blast.ncbi.nlm.nih.gov) to identify matching or closely alike sequences for the *VP7* and *VP4* genotype assignments. The representative partial nucleotide sequences of the *VP7* and *VP4* genes from group A rotaviruses in this study have been put forward into the GenBank with the accession numbers GQ 412143, GQ 412147, GQ 412148, GQ 412149, GQ 412150, GQ 412151, and GQ 412152. The GenBank submitted sequences were phylogenetically analyzed with other universal G and P genotype sequences retrieved from NCBI to determine the relatedness among sequences.

## 3. Results

### 3.1 Detection and prevalence estimation of rotavirus by RNA-PAGE

The RNA-PAGE analysis of dsRNA extracted from fecal samples revealed a characteristic 11-band pattern typical of rotavirus, reflecting its segmented/splitted genome. The overall rotavirus prevalence through RNA- PAGE was 15.2% (33/217) (Table 1). Among the isolates, 27 displayed a long electropherotype, while 4 exhibited a short electropherotype, and 2 showed mixed patterns. All the adult samples were negative for rotavirus by PAGE, whereas prevalence in children was found to be 16.01% (33/206). Further analysis revealed prevalence in diarrhoeal cases to be 18.39% (32/174) and non-diarrhoeal stool samples to be 3.12% (1/32). The gender-based analysis of the data revealed a marginally higher rotavirus prevalence in males 17.85%

**Table 1. Number of samples collected from different regions of India and samples positive for rotavirus by both the detection methods**

| Region | No. of samples analyzed | | | | Total | PAGE positive samples | | | | RT-PCR positive samples | | | | PAGE and/or RT-PCR positive samples | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | D | | ND | | | D | | ND | | D | | ND | | D | | ND | |
| | M | F | M | F | | M | F | M | F | M | F | M | F | M | F | M | F |
| Southern | 24 | 16 | 0 | 0 | 98 | 4 | 1 | 0 | 0 | 3 | 1 | 0 | 0 | 4 | 1 | 0 | 0 |
| | 58* | | | | | 9 | | | | 8 | | | | 12 | | | |
| Western | *15* | 9 | 2 | 3 | 29 | 7 | 2 | 0 | 0 | 6 | 0 | 0 | 0 | 10 | 2 | 0 | 0 |
| NEH | *30* | 22 | 13 | 14 | 79 | 3 | 6 | 1 | 0 | 7 | 2 | 0 | 0 | 8 | 6 | 1 | 0 |
| Total | *69* | 47 | 15 | 17 | 206 | 14 | 9 | 1 | 0 | 16 | 3 | 0 | 0 | 22 | 9 | 1 | 0 |
| | *58** | | | | | 9 | | | | 8 | | | | 12 | | | |

*D- Diarrhoeal; ND-Non-diarrhoeal; M- Male; F- Female; NEH – North-Eastern Hilly*

** Data for gender was not available for 58 samples collected from southern region of India as indicated in table, however, all these were diarrhoeal stool samples from children below 5 years of age.*

*Note – Eleven stool samples (n = 11) collected from adults (including children above 5 years of age) are not presented in the table. However, all the adult stool samples were negative for rotavirus*

(15/84) compared to females 14.06% (9/64) with the western region had a higher prevalence 31.03% (9/29) followed by southern region 14.28% (14/98) and the NEH region 12.65% (10/79).

### 3.2 Prevalence estimation of rotavirus by RT-PCR

The normalized RT-PCR test successfully amplified a 304 bp product from the partial VP7 gene of rotavirus A using the rota1 and rota2 primers (Table 2). The rotavirus prevalence detected by RT-PCR was 12.4% (27/217) (Table 1). The RT-PCR results were similar to those

**Table 2. Primers used for detection and characterization of human rotaviral strains (nt - nucleotide location in primer sequence)**

| S. No. | Gene | Primer name | Primer sequence | Reference |
|---|---|---|---|---|
| 1 | VP7 | Rota1 | GAT CCG AAT GGT TGT GTA ATC CAA T (nt 531 to 550) | (Husain et al. 1995) |
| | | Rota2 | AAT TCG CTA CGT TTT CTC TTG G (nt 824 to 808) | (Husain et al. 1995) |
| 2 | VP7 | Beg9 | GGCTTTAAAAGAGAGAATTTCCGTCTGG (nt 1-28) | (Gouvea et al. 1990) |
| | | End9 | GGTCACATCATACAATTCTAATCTAAG (nt 1062-1036) | (Gouvea et al. 1990) |
| 3 | VP4 | Con2 | ATTTCGGACCAT'ITATAACC (nt 868 to 887) | (Gouvea et al. 1994a) |
| | | Con3 | TGGCTTCGCCATTTLATAGACA (nt 11 to 32) | (Gentsch et al. 1992) |
| **G Types** | | | | |
| 4 | G1 | 9T1-1 | TCTTGTCAAAGCAAATAATG (nt 176 to 195) | (Das et al. 1994) |
| 5 | G2 | 9T1-2 | GTFlAGAAATGAYTTCTCCACT (nt 262 to 281) | (Das et al. 1994) |
| 6 | G3 | 9T-3P | GTCCAGTTIGCAGTGTTAGC (nt 484 to 503) | (Das et al. 1994) |
| 7 | G4 | 9T-4 | GGGTCGATGGAAAATTCT (nt 423 to 440) | (Das et al. 1994) |
| 8 | G5 | FT5 | CATGTACTCGTTGTTACGTC (nt 779-760) | (Gouvea et al. 1994a) |
| 9 | G6 | DT6 | CTAGTTCCTGTGTAGAATC (nt 499-481) | (Gouvea et al. 1994a) |
| 10 | G8 | HT8 | CGGTTCCGGATTAGACAC (nt 273-256) | (Gouvea et al. 1994a) |
| 11 | G9 | 9T-9B | TATAAAGTCCATFGCAC (nt 131 to 147) | (Das et al. 1994) |
| 12 | G10 | ET10 | TTCAGCCGTTGCGACTTC (nt 714-697) | (Gouvea et al. 1994a) |
| 13 | G11 | BT11 | GTCATCAGCAATCTGAGTTGC (nt 336-316) | (Gouvea et al. 1994a) |
| **P Types** | | | | |
| 14 | [P1] | pNCDV | CGAACGCGGGGGTGGTAGTTG (nt 269-289) | (Gouvea et al. 1994b) |
| 15 | [P4] | 2T-1 | CTATTGTTAGAGGTTAGAGTC (nt 474 to 494) | (Gentsch et al. 1992) |
| 16 | [P5] | pUK | GCCAGGTGTCGCATCAGAG (nt 336-354) | (Gouvea et al. 1994b) |
| 17 | [P6] | 3T-1 | TGTTGATTAGTTGGATTCAA (nt 259 to 278) | (Gentsch et al. 1992) |
| 18 | [P7] | POSU | CTTTATCGGTGGAGAATACGTCAC (nt 389-412) | (Gouvea et al. 1994b) |
| 19 | [P8] | 1T-1 | TCTACTTGGATAACGTGC (nt 339 to 356) | (Gentsch et al. 1992) |
| 20 | [P9] | 4T-1 | TGAGACATGCAATTGGAC (nt 385 to 402) | (Gentsch et al. 1992) |
| 21 | [P11] | pB223 | GGAACGTATTCTAATCCGGTG (nt 574-594) | (Gouvea et al. 1994b) |

**Table 3. Seasonal variation of the Rotavirus prevalence in children by RNA-PAGE and/or RT-PCR**

| Disease | Human samples | |
|---|---|---|
| | No. of samples screened | No. of samples positive through RNA-PAGE and/or RT-PCR |
| November - March | 66 | 23 (34.84%) |
| April - June | 128 | 20 (15.62%) |
| July- October | 23 | 1 (4.34%) |
| Total no. of samples screened | 217 | 44 (20.27%) |

obtained by PAGE, showing that all adult samples were negative, while 13.10% (27/206) of children samples were positive for rotavirus. Further analysis revealed a prevalence of 15.51% (27/174) in diarrhoeal cases, with no rotavirus positive samples found in non-diarrhoeal stool samples from children. A gender-based breakdown showed a significantly higher prevalence in males 19.04% (16/84) compared to females 4.68% (3/64), with the highest rotavirus positivity observed in the western zone/region 20.68% (6/29), followed by the southern zone/region 12.24% (12/98) and the NEH region 11.39% (9/79).

### 3.3 Prevalence estimation of rotavirus by RNA-PAGE and/or RT-PCR

The overall rotavirus prevalence in humans was determined using the results from RNA-PAGE and/or RT-PCR. As shown in Table 1, the combined rotavirus prevalence by RNA-PAGE and/or RT-PCR was 20.27% (44/217). The results also indicated that all the adult stool samples were negative by any of the two methods. However, in case of children, the overall prevalence was 21.35% (44/206) with the higher prevalence of 27.38% (23/84) in male children as compared to female children i.e. 14.06% (9/64), especially from diarrhoeal stool samples i.e. 24.71% (43/174) and very rarely from non-diarrhoeal or normal stool sample i.e. 3.12% (1/32). Therefore, the combined prevalence by these two methods increased to 17.34% (17/98), 41.37% (12/29) and 18.98% (15/79) in southern, western and NEH areas of the country, respectively. Maximum cases of rotavirus were detected during winter (i.e. from November to March) which accounts for 34.84% (23/66) cases followed by April to June where the prevalence rate was recorded as 15.62% (20/128). However, only a single case 4.34% (1/23) reported during the rainy season i.e. July to October (Table 3).

### 3.4 Genotypic characterization of the rotavirus isolates

The RNA-PAGE and/or RT-PCR positive samples were further analyzed for P and G genotyping by amplifying the partial length VP4 gene and the whole-length VP7 gene using specific primers, such as Con3 Con2 and Beg9 End9, which yielded expected PCR products of 876 bp and 1062 bp, respectively (Fig. 1a, b) (Table 2). These PCR amplified products were then subjected to two rounds of semi-nested multiplex PCR with genotype-specific primers. The first multiplex PCR aimed to amplify products of 158 bp (G1), 244 bp (G2), 466 bp (G3), 403 bp (G4), and 110 bp (G9), while the second multiplex PCR targeted products of 780 bp (G5), 500 bp (G6), 274 bp (G8), 715 bp (G10), and 337 bp (G11). Similarly, the VP4 gene PCR amplified products were processed through two separate semi-nested multiplex PCRs using type-specific primers. The third multiplex PCR was designed to produce products of 345 bp (P[8]), 483 bp (P[4]), 267 bp (P[6]), and 391 bp (P[9]), and the fourth multiplex PCR yielded expected products of 555 bp (P[5]), 502 bp (P[7]), 622 bp (P[1]), and 314 bp (P[11]) (Table 2).

In this study, G genotyping was successfully performed on 15 fecal samples, while P genotyping was done for 6 samples (Table 4). Amidst G genotypes in children, G1 was most frequent (5 samples) (Fig. 2a), followed by G12 (4), G11 (4), G10 (1), and G2 (1) (Fig. 2b). For P genotypes, P[6] was the most frequent (4 samples) (Fig. 2c), with only

**Table 4. P and G genotyping of human group A rotaviruses (ND - Not done; NEH - North Eastern Hilly)**

| Region | Samples tested | Samples typable | | Samples untypable | | G Genotypes Identified | | | | | P Genotypes Identified | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | G | P | G | P | G1 | G2 | G10 | G11 | G12 | P[4] | P[6] | P[8] |
| Northern Region | Samples from previous study (VPH, IVRI) | 1 (D-C1) | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 |
| | | 1 (D-C2) | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| | | 1 (D-CH9) | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 |
| Southern Region | Hyderabad | 13 | 4 | 3 | 9 | 10 | 2 | 0 | 1 | 1 | 0 | 0 | 3 |
| | Bangalore | 3 | 0 | 0 | 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | Bider | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| Western Region | Navi Mumbai | 7 | 1 | 1 | 6 | 6 | 1 | 0 | 0 | 0 | 0 | 1 | 0 |
| | Mumbai | 5 | 0 | ND | 5 | ND | 0 | 0 | 0 | 0 | 0 | ND | ND |
| NEH Region | Shillong, Meghalaya | 3 | 0 | ND | 3 | ND | 0 | 0 | 0 | 0 | 0 | ND | ND |
| | Gangtok, Sikkim | 12 | 6 | ND | 6 | ND | 0 | 1 | 0 | 1 | 4 | ND | ND |
| | Total | 44+3 = 47 | 15 | 6 | 32 | 21 | 5 | 1 | 1 | 4 | 4 | 1 | 4 |

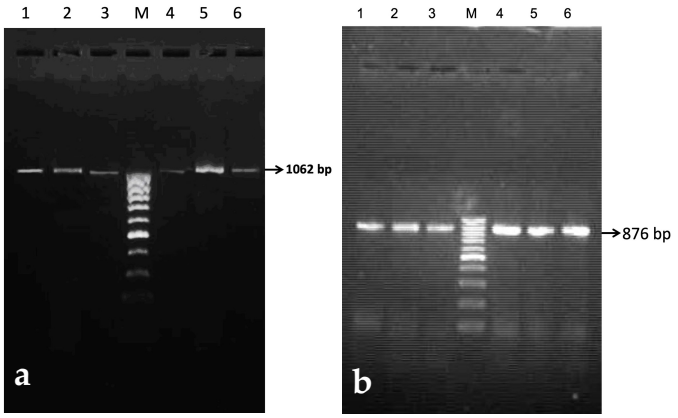


***Fig. 1a.*** *PCR for full length amplification of VP7 gene (1062 bp). Note: Lane M: DNA ladder (100 bp); Lane 1-6: PCR products (1062 bp) of VP7 gene*
***Fig. 1b.*** *Amplification of large segment of VP4 gene (876 bp). Note: Lane M : DNA ladder (100 bp); Lane 1-6 : PCR products (876 bp) of VP4 gene*

one sample each identified for P[4] (Fig. 2c) and P[8] genotypes. Notably, a variety of P and G genotype combinations were noticed,

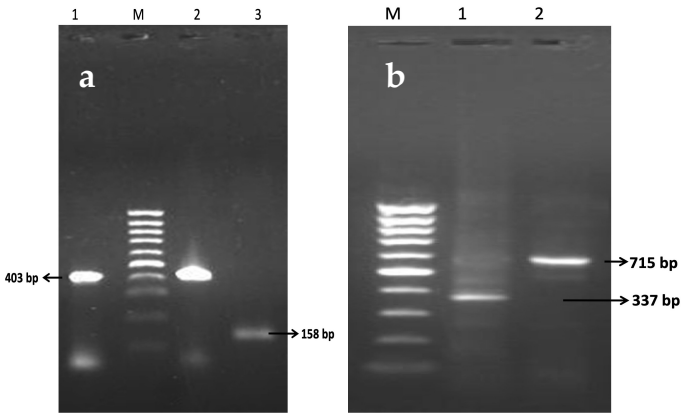


***Fig. 2a.*** *First Multiplex PCR for G genotyping of rotavirus: Lane M : DNA ladder (100 bp); Lane 1 & 2 : PCR product (403 bp) of G4 genotype; Lane 3 : PCR product (158 bp) of G1 genotype*
***Fig. 2b :*** *Second multiplex PCR for G genotyping of rotavirus; Lane M : DNA ladder (100 bp); Lane 1 : PCR products (337 bp) of G11 genotype; Lane 2 : PCR products (715 bp) of G10 genotypes*

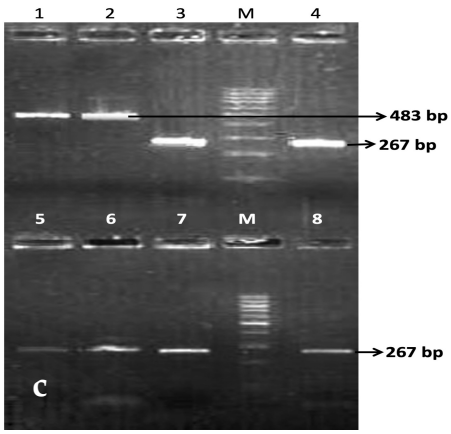


***Fig. 2c.*** *Third multiplex PCR for P genotyping of rotavirus: Lane M : DNA ladder (100 bp): Lane 1 & 2 : PCR products (483 bp) of P[4] genotype (in duplicate): Lane 3-8 : PCR products (267 bp) of P[6] genotype (in duplicate)*

with the G1P[6] combination being the most common among isolates from children. Specifically, G1P[6] was found in two isolates from

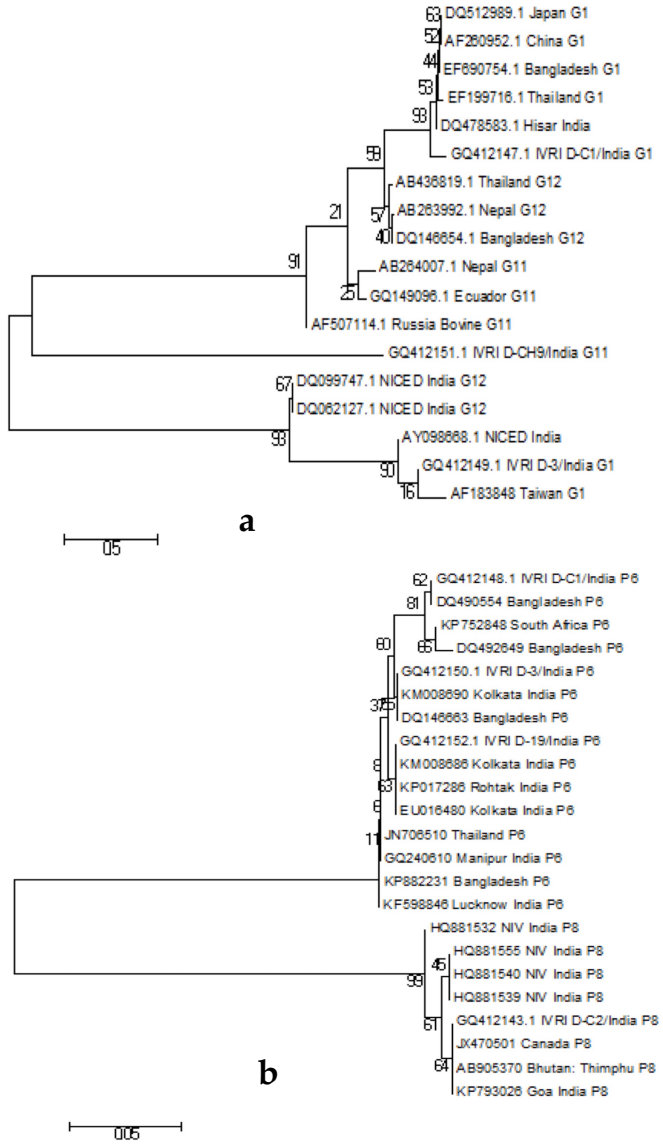


***Fig. 3.*** *Phylogenetic analysis of VP7 (a) and VP4 (b) gene sequences*

Hyderabad and one isolate (D-C1) from Bareilly. Only one sample showed G1P[4], collected from Mumbai and two isolates showed rare combinations i.e. G11P[8] from Bareilly and G11P[6] from Hyderabad. Besides these, four G12 genotypes from Sikkim and one each of G10 from Hyderabad, G11 each from Sikkim and Bareilly were also detected.

### 3.5 Sequencing of the isolates

Seven PCR products were further confirmed by nucleotide sequencing (accession nos. GQ 412143, GQ 412147, GQ 412148, GQ 412149, GQ 412150, GQ 412151 and GQ 412152). The results indicated that the genotypes identified through RT-PCR using genotype-specific primers were found to be the same as that by nucleotide sequencing. Most of the sequences exhibited a high degree of similarity/close relatedness with rotavirus strains from India and Bangladesh submitted to the GenBank (Fig. 3).

## 4. Discussion

In the present study, an overall prevalence of 20.27% (44/217 samples) was observed for human group A rotaviruses. Various studies accompanied across different zones/regions of India have reported

varying prevalence rates of rotavirus in the stool/faecal samples from hospitalized children below 5 years of age with acute gastroenteritis (Kelkar et al. 1999; Jain et al. 2001; Broor et al. 2003; Kaur et al. 2008; Malla et al. 2022). It is well-established that male children are more susceptible to rotavirus infections than female children. Alike pattern was noticed in the present study, where the prevalence in males (71.87%) was higher than in females (28.12%). Previous studies have also indicated an elevated prevalence of rotavirus infections in male children compared to female children (Kelkar et al. 1999; Elawad et al. 2015; Pol et al. 2017; Lafta et al. 2019). None of the samples from adults was found positive for rotavirus by any of the two methods followed in the present investigation which is indicative of the development of immunity. Infant age of 6-12 months was thought to be the most susceptible period due to a decrease in the maternal antibodies against the virus which offers protection up to 4 months and later after 12 months some acquired immunity also comes into play (Kelkar et al. 1999).

Though the rotavirus is present even after diarrhoeal episodes, it is commonly and easily detected from diarrhoeal stool samples because of a strong association of rotavirus with diarrhoea. A higher rate of rotavirus positivity was detected in diarrhoeic stools (24.71%) as compared to normal stools (3.12%) in the present study. Similar findings of higher positivity in diarrhoeal cases than non-diarrhoeal cases was also observed in the previous studies (Sherchand and Haruki 2004; Abass et al. 2021). As the name winter diarrhoea, the prevalence is generally higher in winter (i.e. December, January, and February) (Sherchand and Haruki 2004; Raorane et al. 2020; Malla et al. 2022) which we also observed during an epidemiological survey of rotavirus where maximum cases i.e. 34.84% (23/66) occurred during winter (i.e. from November to March). However, some reports indicated the prevalence of rotavirus in the summer and rainy season also. This is because of longer survivability and stability of rotavirus even at a high temperature which allows them to remain infectious in the environment (Kurmi et al. 2012). Depending on these factors, the prevalence of rotavirus is varied from place to place. We also reported a higher prevalence of rotavirus from western India (40.0%) followed by southern India (17.34%). This may be due to the simultaneous use of two tests *viz*. PAGE and RT-PCR for rotavirus detection in the faecal samples which maximized the detection levels. As seen from our results, there were 6/30 (20.0%) and 3/20 (15.0%) stool samples which were exclusively positive by RNA-PAGE and RT-PCR, respectively from the western region whereas 12/30 (40.0%) samples turned out to be positive by any of these two methods. Previous studies have also highlighted the advantage of using multiple diagnostic tests to improve detection rates (Hussain et al. 1995). RNA-PAGE in present investigation alone detected rotavirus in 15.2% (33/217) of the samples, with most exhibiting a long electrophoretic pattern, four showing a short pattern, and two demonstrating mixed patterns (more than 11 bands). A similar dominance of the long pattern has been reported in other works taken previously (Kaur et al. 2008; Broor et al. 1993; Dubal et al. 2013; Raorane et al. 2020). Mixed infections were also observed in previous studies (Minakshi 1999), and the presence of extra viral RNA segments suggests either simultaneous or sequential infections by multiple electropherotypes or changes in the RNA segment length during infection (Minakshi 1999). Similarly, the other test i.e. RT-PCR targeting *VP7* gene by Rota 1 and Rota 2 primers could detect 12.4% (27/217) cases of rotavirus infection, which was much less than the actual overall prevalence of 20.27% and also less than that detected by RNA-PAGE (15.2%). Despite RT-PCR's higher sensitivity, capable of detecting as few as $10^4$ rotavirus particles per mL compared to the $10^{11}$ particles required for RNA-PAGE (Arguelles et al. 2000), sequence variations in the viral genome complicate the design of universal primers for all rotavirus types (Steele et al. 1995). Several RNA-PAGE positive samples of the present study showed negative results by RT-PCR. A similar observation of lower RT-PCR positivity compared to RNA-PAGE has been reported in both human and bovine samples (Wani et al. 2004). Non-specific inhibitors of PCR in fecal samples may account for the low RT-PCR positivity (Xu et al. 1990; Wani et al. 2004). Husain et al. (1995) also noted reduced sensitivity of RT-PCR assay compared to PAGE when RNA was extracted using phenol and ethanol precipitation due to the presence of inhibitors. Purifying RNA over CF11 cellulose removed these inhibitors, improving sensitivity. Other methods have also been used to eliminate these inhibitors (Xu et al. 1990; Gentsch et al. 1992). Moreover, 11 samples came out positive by RT-PCR but were negative by RNA-PAGE, which can be ascribed to RT-PCR's higher sensitivity (detecting $10^4$ viral particles) in comparison to RNA-PAGE (detecting $10^{11}$ viral particles) (Arguelles et al. 2000).Thus, our findings suggest that using multiple methods simultaneously can maximize diagnostic potential and improve detection efficiency.

In the current study, the G1 genotype was established to be the most dominant, followed by G12, G11, G10, and G2. Previous research in India has reported the presence of genotypes such as G1, G2, G3, G4, G6, G8, G9, G10, and G12 in the human population (Broor et al. 2003; Kang et al. 2005; Ramani and Kang 2007; Raorane et al. 2020), with G1 being the most common, leading 22-57% of cases (Chitambar et al. 2014; Pradhan and Chitambar 2018). Other genotypes like G10, G11 and G12 recovered in this study warrant further investigation because G11 genotype isolated earlier from human fecal samples shares an evolutionary lineage with porcine strains (Rahman et al. 2005a) or has been identified as a swine- specific pathogen exclusively (Timenetsky et al. 1997). Similarly, the G6 and G10 genotypes have been primarily observed in bovine populations (Minakshi 1999; Wani et al. 2004). Although the G2 genotype was noted in an earlier study involving rotavirus-positive samples from patients at Assam Medical College in Dibrugarh, Assam (NICED, Annual Report 2002-03), G11 has not previously been reported in the North Eastern Hill (NEH) region. The G12 genotype, first reported in Manipur in 2010 (Mukherjee et al. 2010), was actually detected slightly earlier, in 2008-09, in samples from Sikkim in this study. Additionally, G1 and G12 were found to be the predominant serotypes in Nepal (Uchida et al. 2006). With regard to P genotypes, P[6] was the most commonly detected strain in this study, appearing in seven samples, followed by one sample each of P[4] and P[8]. In India, the most prevalent P genotypes were P[8] (27%), P[6] (21%), and P[4] (20%) (Rahman et al. 2007). Sequence analysis of selected P[6] strains revealed that the D-3, D-18, and D-19 isolates exhibited 100% homology with the Dhaka strain (Access. No. DQ146663), indicating a significant circulation of this strain from Dhaka, Bangladesh, to Hyderabad, India.

In India, uncommon genotypes for instance G1P[6] and G4P[6] were identified in 43% of the tested strains (Ramchandran et al. 1996), alongside other genotypes like G1P[4], G9P[6], G3P[6], G3P[8], G1P[11], G1P[10], and G9P[8] in children (Bahl et al. 2005; Ayyash et al. 2009). Similarly, the current study also found G1P[6] to be the most prevalent genotype in Hyderabad, while one sample from Mumbai displayed G1P[4]. Additionally, two samples exhibited rare combinations viz.

G11P[6] from Hyderabad and G11P[8] from Bareilly. Previous reports have also mentioned the presence of G11, G11P[6], G12P[6], and G11P[8] genotypes in various locations (Hussain et al. 1993; Rahman et al. 2005b; Rahman et al. 2007; Banyai et al. 2009; Bwogi et al. 2023). The *VP7* partial gene sequences of the three G11 rotavirus strains in this investigation exhibited close similarity to the porcine-like G11 strain from Dhaka (Rahman et al. 2005b).

## 5. Conclusions

Rotaviruses have been discovered in 20.27% of the samples through PAGE and/or RT-PCR, with G1P[6] being the most predominant genotype. One sample exhibited the G1P[4] combination, while two others showed the G11P[6] and G11P[8] combinations. Ongoing molecular epidemiological investigations are essential to find out whether the G11P[6] and G11P[8] genotypes are becoming dominant in India and to explore the genetic material exchange among porcine, bovine, and human rotavirus strains. The emergence of such uncommon or novel strains may necessitate updates to existing anti-rotaviral vaccine components. Consequently, comprehensive molecular epidemiological research is needed in both humans and also in animals, to better understand the potential transmission dynamics of rotavirus between these populations.

## Declarations

**Funding**: This research was funded by grants from the Institute project, provided by ICAR-Indian Veterinary Research Institute, Izatnagar

**Conflict of interest**: The authors declare that they have no conflicts of interest or competing interests of any kind

**Acknowledgements**: The authors would like to express their gratitude to the Director and Joint Director (Research) of the Indian Veterinary Research Institute, Izatnagar, India, for providing the essential resources for this study. The authors also extend their gratitude to the hospital administrators and the parents of the children for allowing the sample collection

**Ethics approval and consent of participation**

In this study neither laboratory animal experiments nor any invasive procedures were performed on humans during stool sample collection. Hence, no ethical approval was needed. Moreover, informed consent was acquired from each child's guardian and from the administrators of childcare hospitals

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## Citation

Dubal ZB, Malla BA, Bhilegaonkar KN, Sailaja VV, Pal P, Kapil J, Rawat S, Malik YPS. (2025). Rotavirus prevalence with G and P genotypes in different regions of India. Letters in Animal Biology 05(2): 41 – 49.